

# A Prospective Pilot Study on the Clinical Application of Stromal Vascular Fraction Stem Cells in the Treatment of Miller Class I and II Gingival Recession Defects

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## Abstract



**R**egenerative medicine is a rapidly expanding set of innovative technologies that restore function by enabling the body to repair, replace, and regenerate damaged, aging or diseased cells, tissues and organs. In the practice of surgical dentistry, a number of products and techniques have been introduced and used over the past 20 years to stimulate regenera-

tion of dental tissues including bone and gingiva. One such technique which has recently been used for such purposes is the use of adult multipotent cells obtained from adipose tissue. The aim of this prospective pilot study case series is to report use of the IntelliCell™ adipose processing technique to obtain adult multipotent cells for use in the treatment of gingival recession.

**KEY WORDS:** Adipose multipotent cells, IntelliCell,™ gingival recession, gingival grafting

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## INTRODUCTION

Regenerative medicine is a rapidly expanding set of innovative medical technologies that restore function by enabling the body to repair, replace, and regenerate damaged, aging or diseased cells, tissues and organs. In the practice of surgical dentistry, a number of products and techniques have been introduced and used over the past 20 years to stimulate regeneration of dental tissues including bone and gingiva. One such technique which has recently been used for such purposes is the use of adult multipotent cells obtained from adipose tissue. The aim of this prospective pilot study case series is to report use of the IntelliCell™ adipose processing technique to obtain adult multipotent cells for use in the treatment of gingival recession.

In the case of the Intellicell™ technique, one can harvest 60 cc of adipose (fat) tissue from a patient's stomach, hips or outer or inner thighs to produce a variety of growth factors for use in surgical dentistry. With this technique, harvested adipose tissue is processed using ultrasonic cavitation to produce stromal vascular fraction (SVF). The cellular composition of the SVF ranges from adult stem cells (Mesenchymal Stem Cells), pre-adipocytes, endothelial cells, smooth muscle cells, pericytes, fibroblasts and growth factors. Typically, the SVF also contains blood cells from the capillaries supplying the fat cells. These include erythrocytes, B and T cells, macrophages, monocytes, mast cells, natural killer (NK) cells, hematopoietic stem cells and endothelial progenitor cells, to name a few. Hematopoietic stem cells and endothelial progenitor cells play important roles in supporting the viability

of existing blood vessels and faster healing.

The IntelliCell™ technology process yields SVF which is a functionally diverse cell population of cells that it is believed to be synergistic and able to communicate with other cells in their local environment. The mechanism of action of the SVF is more than regenerative. The mixtures of cells in SVF have multiple functions that are highly integrated and may be more potent than the adipose stem cells themselves. The IntelliCell™ technology process yields autologous and homologous stromal vascular fraction. Under FDA 361 published laws, the cells produced must be autologous, minimally manipulated, used during the same procedure, and must be homologous. Once the cells are returned to the doctor, and in this case the periodontist, the cells can be used off label.

The SVF have the following regenerative cell function properties: Anti-inflammatory/Immunomodulation, Trophic Support Differentiation, and Homing. The Anti-inflammatory/Immunomodulation properties have been shown to suppress mixed lymphocyte reactions and inhibit T cell proliferation induced by third cell type mitogenic factors. These cells have been shown to be able to control lethal graft versus host disease (GVHD) in mice after haploidentical hematopoietic transplantation. The Trophic Support of these cells have been demonstrated to secrete a number of angiogenesis-related cytokines such as: Vascular endothelial growth factor (VEGF), Hepatocyte growth factor (HGF), Basic fibroblast growth factor (bFGF), Granulocyte-macrophage colony stimulating factor (GM-CSF), and Transforming growth factor –  $\beta$  (TGF- $\beta$ ). The differentiation quality of these cells is due to the fact that they con-

tain stem cells which studies demonstrate a diverse plasticity, including differentiation into adipo-, osteo-, chondro-, myo-, cardiomyo-, endothelial, hepato-, neuro-, epithelial and hematopoietic lineages. In vivo experiments and functional studies have demonstrated the regenerative capacity of IntelliCells to repair damaged or diseased tissue via transplant engraftment and differentiation. Awad and colleagues<sup>1</sup> reported significant improvements using autologous MSC delivery in a rabbit Achilles tendon repair model compared to cell-free collagen control rabbits. Nixon et al.<sup>2</sup> demonstrated statistically significant improvement in histological repair of a collagenase induced injury in the superficial digital flexor tendonitis in horses treated with IntelliCells harvested from fat. Cowan and colleagues<sup>3</sup> demonstrated that IntelliCells heal a critical-size mouse calvarial defect in which there was increased bone formation and mineralization compared to controls. Jeong et al.<sup>4</sup> demonstrated in a rodent cerebral infarct model, that infarcted rats administered magnetically labeled IntelliCells administered two weeks after the creation of an infarct experienced restoration of locomotor function compared to controls. Homing is an important function of IntelliCells™ which is the mechanism by which a cell migrates from one area of the body to a distant site. Homing is an important function of IntelliCells™ and other progenitor cells and one mechanism by which intravenous or parenteral administration of IntelliCells™ permit cells to effectively target a specific area of pathology. Nilsson et al.<sup>5</sup> demonstrated that labeled cells of bone lineage injected intravenously into mice can engraft, form bone, and give rise to osteocytes and bone lin-

ing cells detectable on the mouse femur.

Apoptosis is defined as a programmed cell death or “cell suicide”, an event that is genetically controlled. Under normal conditions, apoptosis determines the lifespan and coordinated removal of cells. Unlike necrosis, apoptotic cells are typically intact during their removal (phagocytosis). Rehman et al.<sup>6</sup> demonstrated anti-apoptosis in acutely injured tissue denied critical blood-flow resulting in ischemia. IntelliCells significantly reduced endothelial cell apoptosis.

Gingival recession, with well-known negative sequelae, including cervical caries, dentinal root sensitivity, difficulty in allowing adequate plaque control, and esthetic deficiencies, demands effective surgical intervention with minimum intra-operative and post-operative complications. In recent years, a number of systematic reviews have examined clinical outcomes of various surgical approaches to recession defects, including the coronally advanced flap (CAF) alone, CAF in combination with the subepithelial connective tissue graft (CTG), guided tissue regeneration (GTR), enamel matrix derivative (EMD), and acellular dermal matrices (ADM).<sup>6-11</sup> Although current alternatives to CTG + CAF appear effective when examining specific clinical parameters, only CTG + CAF appears consistently effective in relation to all clinical efficacy endpoints, especially long-term maintenance of root coverage.<sup>12-26</sup> Although such evidence based data has led many clinicians to view the CTG as the gold standard treatment in reducing or eliminating gingival recession, disadvantages such as the need for a distant donor site, increased morbidity associated with graft harvest, and limited amounts



**Figure 1:** A mini liposuction is completed to harvest fat for stem cell processing.



**Figure 2:** The fat is harvested and prepared for processing.

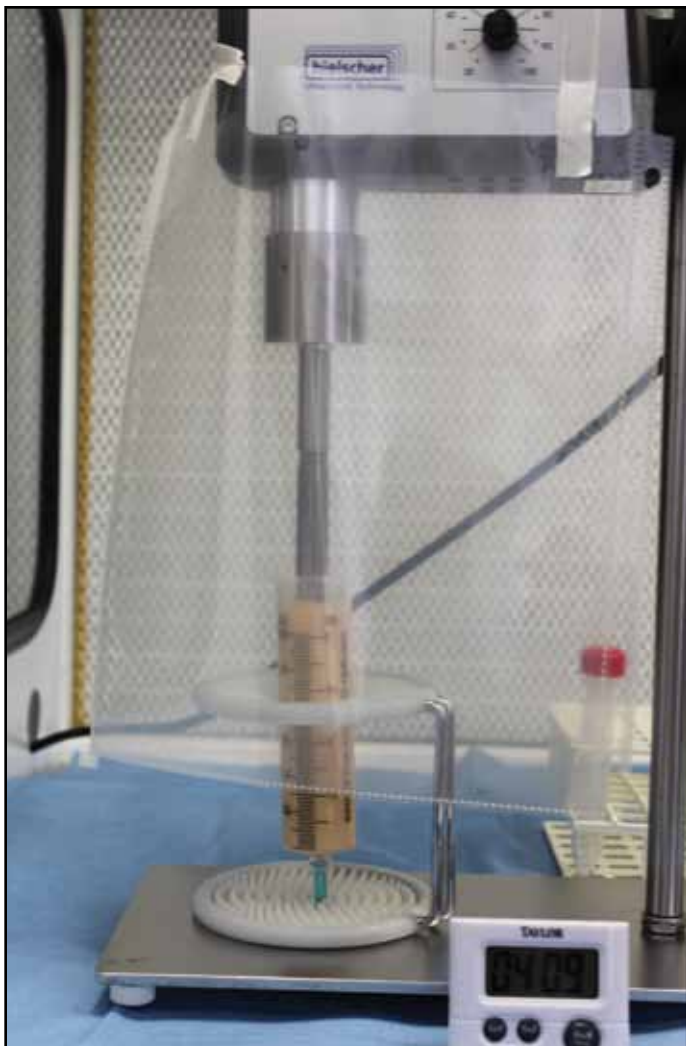


**Figure 3:** The fat is processed via IntelliCell Biosciences technique.

of available donor tissue have continued to stimulate the search for comparably effective, alternative therapies to autologous grafts.<sup>27,28</sup>

Recently, a 510(K)- FDA cleared xenogenic porcine bilayer collagen matrix (CM) (Mucograft®, Osteohealth Company, Shirley, NY) composed of pure type I and III collagen obtained by standardized, controlled manufacturing processes without further cross-linking or chemical treatment has been cleared for multiple regenerative indications, including treatment of gingi-

val recession defects around teeth. Designed to support tissue ingrowth and regeneration, CM's enhanced bilayer thickness facilitates surgical manipulation, provides support for mucosal cellular migration and regeneration, and supports clot stabilization and subsequent soft and hard tissue ingrowth.<sup>29-34</sup> A number of recently published prospective clinical trials investigating CM's efficacy in treating both keratinized mucosal deficiencies and gingival recession defects suggest that CM may provide a viable alternative to autogenous soft tissue grafts as well as to other currently favored approaches.<sup>32-35</sup> With the advancements of stem cell regenerative medicine the authors decided to use SVF containing adult adipose stem cells and combine it with xenogenic porcine bilayer collagen matrix as a replacement for the traditional mucous membrane roof graft. The following consecutive case series study was designed to further investigate the use of SVF containing adult adipose stem cells when combined with potential xenogenic porcine bilayer collagen matrix in the treatment of root recession.



**Figure 4:** Ultrasonic cavitation of adipose fat to manufacture stromal vascular fraction IntelliCell BioSciences technique.

## MATERIALS AND METHODS

### Subject Population

Eight healthy patients, 7 female and 1 male, ranging from 23 to 45 years of age with Miller Class I or II gingival recession defects of  $\geq 3$  mm, with a mean defect depth of 3.79 mm, were included in this prospective consecutive case series study. The majority of patients were non-smokers, although a number of patients at the time of treat-



**Figure 5:** After centrifugation to separate fat from cells and form pellet.



**Figure 6:** Removing cellular pellet after centrifuging.

ment smoked up to 5 cigarettes per day. Many of the patients presented with multiple contiguous gingival recession defects, although single defects were also included. Of the 8 cases, 5 were treated in the maxilla and 3 in the mandible. In all treated defects, a minimum of 1 mm of marginal keratinized gingiva was present at initiation of treatment. All patients were treated in a single private practice office in Manhattan, New York.



**Figure 7:** The specimen is placed in a flow cytometer to measure cells.



**Figure 8:** Please note the cellular pellet at the bottom.



**Figure 9:** The stem cells are ready for delivery into the surgical site.



**Figure 10:** The stem cells are loaded into a syringe.

### Clinical Evaluation

At the initial visit, the defect sites were examined clinically, photographic documentation performed, and defect measurements recorded. Following the initial baseline screening and surgery, all patients were followed for a minimum of 6 months. Radiographs were taken at baseline and at week 24. The following baseline clinical parameters were recorded for each consecutively treated patient: gingival recession depth; height of keratinized tissue from



**Figure 11:** The preoperative Miller 2 recession prepped for incisions.



**Figure 12:** Elevation of full thickness flap.



**Figure 13:** Mucograft is injected with the stem cells and sutured in place.



**Figure 14:** More stem cells are injected into the surgical area prior to flap closure.

the free gingival margin to the mucogingival margin; buccal and proximal probing pocket depths; and degree of gingival inflammation. At six months post-surgery, photographic documentation was again obtained as were the clinical parameters measured at baseline.

### **Surgical Procedure**

Prior to surgery, all subjects received oral hygiene instructions, received full-mouth prophylaxis, and were not appointed for sur-

gery until they were capable of demonstrating adequate supra-gingival plaque control.

On the day of surgery, the patient underwent liposuction by a licensed physician and the fat which was obtained and was processed by the IntelliCell™ process (Figs. 1-10). This process consisted of taking 60cc of the lipoaspirate which was subjected to ultrasonic cavitation as per the proprietary IntelliCell™ BioScience protocol. The resulting product was filtered, washed and centrifugated to cre-



**Figure 15:** Three months postoperative view with up to 100 percent root coverage.



**Figure 16:** Case #2 presurgical view of maxillary Miller 2 recession.



**Figure 17:** Case #2 surgical site with stem cells and Mucograft in place.



**Figure 18:** Case #2 three months after surgery.

ate a pellet of stromal vascular fraction (SVF). The SVF was withdrawn using a 20 cc syringe. A sample of the SVF was then tested using a Millipore Guava flow cytometer to test for cell count, viability, dead cells and debris. The patients averaged 12 Million lived cells per milliliter. A total of 3cc of SVF cells were provided to the periodontist from the physician.

At surgery, local anesthesia was administered and a reversed bevel intrasulcular incision without vertical releasing incisions was made. A

full-thickness mucoperiosteal flap was reflected apically to the mucogingival junction, followed by an apical partial thickness dissection (figure 12) to eliminate muscle tension and to facilitate coronal repositioning of the flap. The root surfaces were then planed and recontoured by odontoplasty in order to assure root confinement within the surrounding alveolar housing. In addition to hand instrumentation, rotary finishing burs or ultrasonics with diamond-coated inserts were used to perform the odontoplasty.



**Figure 19:** Case #2 three months after surgery (secondary view).

The root surfaces were then decontaminated with tetracycline paste to eliminate the bacterial smear layer. The buccal portions of the interdental papillae were then de-epithelialized to create a connective tissue bed for subsequent suturing of the coronally advanced flap. Mucograft® collagen matrix was then properly sized, positioned to cover the exposed roots (figure 13), allowed to saturate with the fibroblast stem cells (figure 14) provided via the liposuction, and sutured to the interdental papillae. (Figure 4) The fully mobile flap was then coronally advanced with minimal to no tension to the level of the CEJ and sutured with 5.0 vicryl suture to the de-epithelialized surfaces of the interdental papillae. Care was taken to avoid compression of the collagen matrix graft. Post suturing the fibroblast stem cell matrix was injected into and around the surgical area.

Patients were instructed not to brush the teeth in the treated area but to use chlorhexidine (0.2%) mouth rinse twice daily the first two weeks. During the next two weeks patients were instructed to apply chlorhexidine with a cotton

swab to the treated areas. Following this period patients were instructed in a brushing technique that avoided apical directed toothbrush trauma to the surgerized segments. At six months after surgery, patients were re-evaluated according to the aforementioned protocol (figure 15).

## RESULTS

### Patient Centered Results

During the six-month follow-up period no significant treatment related adverse events occurred. Immediate post-operative swelling, inflammation and discomfort were minimal. For all treated areas the color, texture and tissue thickness at month six appeared indistinguishable from the adjacent anatomic areas. Figures 16 – 19 depict one of the eight patients of this prospective pilot study.

### Objective Clinical Results

The mean root coverage gain at the end of six months was 3.00 mm, with a mean percent root coverage gain of 86.83%. The mean residual recession depth was 0.79 mm. 66% of the 8 patients experienced 100% root coverage. At the end of the 6-month follow-up period, a mean gain of 0.70 mm of marginal keratinized tissue was realized.

## DISCUSSION

McGuire in 2008 showed tissue-engineered graft bilayered cell therapy (BCT) containing fibroblast stem cells was safe and capable of generating de novo Keratinized tissue (KT) without the morbidity and potential clinical difficulties associated with donor-site surgery.<sup>34</sup> The amount of KT generated with free gingival graft was greater than generated with BCT; however,

24 of 25 test sites demonstrated an increase in KT at 6 months, with more than three-quarters of the sites yielding  $>$  or  $=$ 2 mm bands of KT. In an attempt to reduce surgical morbidity secondary to graft harvest as well as to avoid inherent autogenous tissue supply limitations, alternatives to the CTG continue to be desired goals in the treatment of gingival recession defects. The current case series, when examined along side other recently published studies, provides additional insight into the utility and efficacy of Stromal Vascular fraction (SVF) containing adult adipose stem cells the porcine derived collagen matrix, Mucograft<sup>®</sup>, as an alternative to the CTG and acellular dermal matrices for the treatment of gingival recession defects.

In a recent 6 month prospective, randomized, split-mouth designed clinical trial McGuire et al.<sup>34</sup> compared Mucograft<sup>®</sup> to palatal CT grafts in the treatment of Miller Class I and II recession defects. On multiple parameters, including recession depth, percent root coverage, width of keratinized tissue, color and texture of treatment sites, and subject esthetic satisfaction, Mucograft<sup>®</sup> and SVF proved a viable alternative to the CTG. When patient centered outcomes were considered, Mucograft<sup>®</sup> and SVF were particularly attractive in eliminating the need for an invasive harvesting procedure. The results of the current study compare favourably to this randomized prospective study, with comparable gains in mean root coverage gain and percent root coverage. It was also noted that these patients often reported little to no pain or swelling post surgically.

Although the current case series suggests Mucograft<sup>®</sup> with SVF may be a viable and advantageous alternative to treating gin-

gival recession defects, further randomized controlled clinical trials with increased patient enrolment and follow-up times are needed. Additionally, studies examining what the potential effects of adding SVF for the use of periodontal regenerative therapies will be instructive in clearly defining the range of clinical indications for the use of SVF in dental practice.●

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**Disclosure**

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